MT ends that contact the barrier start to shrink with a speed slightly slower that the speed of dynein, which is much slower than the shrinkage speed of free MTs, occasionally switching back to a growing state. These results suggest that, on its own, dynein attached to a surface can capture and stabilize dynamic MT plus ends.

162-Wkshp Single-Molecule Studies of the Eukaryotic RNA Polymerase II

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Single-molecule methods allow for the real-time analysis of movement and conformational changes of proteins, nucleic acids as well as protein-nucleic acid complexes. Therefore, in contrast to other structural methods such as x-ray crystallography these methods allow for the direct visualisation of flexible domains within large multiprotein/nucleic acid complexes.

I will present a novel hybrid method of high-resolution crystallographic data and single-pair FRET measurements which enables us to find a previously unknown position of a flexible domain within a large complex whose overall structure is well known. This method is applied to elongation complexes of yeast RNA polymerase II, helping us to determine the exit pathway of the nascent RNA.

163-Wkshp Biomolecular Confinement, Mixing, and Interactions in Hydrosomes

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To observe the dynamics or kinetics of individual molecules or molecular complexes on timescales longer than 1 ms, it is often necessary to confine the molecule to an observation region. Techniques previously used to confine or immobilize molecules or complexes include surface attachment, gel encapsulation, and more recently liposome encapsulation [1]. Water-in-oil emulsions have been used to sequester biomolecules into discrete microdroplets; the first measurement of single enzyme activity was demonstrated in this way [2]. We introduce the use of optically trappable aqueous nanodroplets (hydrosomes) [3] for isolation, confinement and study of individual biomolecules and biomolecular complexes. Hydrosomes fuse on contact, which facilitates assembly of separate moities to form a molecular complex. In this talk I will describe techniques for generating hydrosomes and the fluorescence detection of single molecules confined in them. A comparison between single molecule-pair fluorescence resonance energy transfer (spFRET) data taken on a surface and in hydrosomes will be shown. The results of fluorescence polarization anisotropy lifetime measurements of water soluble proteins and nucleic acids in hydrosomes will be discussed; we find these molecules can be freely rotating in this confining environment. We use hydrosomes to confine and study transiently interacting molecular complexes on an individual basis. For example, building upon previous work [4], we use spFRET to study the reaction between the serpin alpha-1 protease inhibitor and the protease rat trypsin.

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Workshop 3: Structural Genomics: A Discussion

164-Wkshp Structure Genomics: An Integral Partner with Functional and Chemical Genomics for Biology and Medicine

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Just as "traditional" structural biology seeks to understand protein biochemical function via integration of structural knowledge with biochemical, biophysical and other functional data, structural genomics offers the opportunity to integrate these types of data in the context of entire genomes or protein families.

I will present examples in which structural coverage of entire protein families combined with enzymatic and/or binding specificity screens has provided functional and mechanistic information that could not have been obtained from a traditional one-protein-at-a-time approach. By using the hundreds of purified proteins generated in structural genomics we are able to

1. identify compounds that promote protein purification or crystallization,
2. identify potential inhibitors or other modulatory molecules, and
3. identify potential substrates for orphan members of gene families,
4. compare substrate or ligand specificity across a family of related proteins, and
5. map out mechanistic details of enzymatic activity by integrating structure and activity profiles for an enzyme family.

Data will be presented from The Structural Genomics Consortium (www.theSGConline.org), an international charity which focuses on human and malarial proteins, and the Northeast Structural Genomics Consortium (www.NESG.org), part of the PSI-2 which focuses on eukaryotic proteins.

165-Wkshp Structural genomics - genome inspired and enabled structural biology

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The Midwest Center for Structural Genomics (MCSG) has established a protein structure determination pipeline using x-ray crystallography and synchrotron radiation. The pipeline addresses a number of bottlenecks in protein crystallography: protein expression, solubility and purification, production of prokaryotic and eukaryotic proteins, HTP crystallization using nanoliter technology and improving crystals and structure determination. We systematically analyze genomic sequences to identify protein families and select targets for structural studies. The MCSG applies the pipeline to protein targets with no structural homologs, biomedical targets and proteins proposed by the community. Using extensively tested methods we generate high-quality protein expression strains, produce milligram quantities of proteins and x-ray quality crystals for structure determination. The cryoprotected crystals are used for data collection at the synchrotron beamlines and structures are determined using semi-automated approaches. Structural models are auto-built and structures are refined, verified and analyzed using semi-automated computational tools. All structures are annotated for function (ProFunc) and ligand binding (GPSS). Results and data are made available to scientific community. The current pipeline capacity is >180 protein structures per year and can process protein targets of considerable difficulty. Advances in the structural genomics pipeline when combined with data collection synchrotron facilities and advanced software and computing resources, have helped to rapidly determine structures of a large number of novel proteins, improve efficiency and lower cost. Using this pipeline, the MCSG has determined over 700 structures including structures of biomedically important classes of proteins from human pathogens. Contribution to genomic and structural biology will be discussed and several examples will be presented.

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166-Wkshp The PSI Structural Genomics Knowledgebase

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The Protein Structure Initiative (PSI) has been successful in producing over 2500 protein structures in a high throughput manner. The strategies used for target selection by PSI centers have resulted in the determination of significantly more novel structures than from structural biology in general. In addition, new technologies for all aspects of the structure determination and analysis pipeline have been developed.

The PSI Structural Genomics Knowledgebase (PSI SG KB) is designed to turn the products of the PSI effort into knowledge that is important for understanding living systems and disease. The PSI SG KB Portal allows access to the structures, annotations, technologies, and models that are the products of the PSI. A description of how this resource will enable further biological discovery will be given.

The PSI SG KB is funded by the NIH.

167-Wkshp Unique Insights into Membrane Protein Biophysics through Structural Genomics

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Structural genomics has provided a unique opportunity to express, isotopically label, purify and prepare NMR samples for a large number of membrane proteins. Indeed, we have developed primary and secondary protocols that express more than 80% of cloned proteins. Ligation independent cloning has proven to be a great asset for testing the expression of many vectors, constructs and mutants. Expression conditions and changes in the vector can be used to shift expression from inclusion bodies to the membrane fraction. Potentially, as many as 20% of the expressed proteins appear in some form of proliferated membrane generating the potential for purification schemes without the use of detergents or denaturants.

Consistent with several other studies we have found that in many cases the water-soluble and membrane-soluble domains can be characterized independently. We have observed specific ion binding, channel conductance, and ligand induced domain folding in isolated domains consistent with studies of the corresponding full length proteins. This suggests that a divide and conquer strategy may be appropriate in many instances for the characterization of these proteins.

From studies of both full length membrane proteins and peptides it is clear that transmembrane helices are often uniform structures resulting from the hydrophobic amino acid composition, low dielectric environment and the lack of water. From solid state NMR observations of resonance patterns, known as PISA wheels, we show that the maximum range of backbone torsion angles is only +/−6 degrees. The fact that these resonance patterns have now been observed in monomeric proteins with up to 7 transmembrane helices and oligomeric proteins with as many as 24 transmembrane helices suggest that the scatter in torsion angles observed in modest resolution x-ray crystallographic results is induced by errors associated with the resolution.

168-Wkshp Structural Genomics, From Proteins To Complexes

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The majority of structural genomics centers were originally mainly concentrated on the set up of high through put facilities for the structure determination of soluble 'globular' proteins. Some of these centers have been very successful with hundreds of new structures determined. Structural genomics has received strong support in Europe, but the EC projects were organized in a different way and have a somewhat different scope from those in the US, Canada or Japan. European projects concentrated on structure determination of challenging, biologically important protein targets. Membrane proteins and multi-protein complexes are highly underrepresented in structural data banks due to tremendous difficulties in preparing sufficient amounts of material for structural analysis. Our group is participating to two important European projects on the structure determination of protein complexes. The first, 3D repertoire, concerns the structural analysis (X-ray, electron microscopy) of multi-protein complexes from yeast. The second (SpineII) targets human complexes involved in medically important cellular processes. Outline and some results of both projects will be presented.

Protein Conformation

169-Pos Solvent-induced Conformation Changes In Short Alanine-rich Peptides

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Board B1

Alanine-rich peptides, with sequences such as Ac-$(AAAAK)_n$Y-NH_2, are useful tools and models for investigating protein/peptide secondary structure transitions. In water, conformation is length dependent; peptides with n greater than or equal to 4 are largely helical in aqueous solution. Exposure to fluorinated alcohols can both stabilize helix formation and modulate backbone-solvent hydrogen-bonding [1]. Alternatively, there is little known about how these solvents affect the conformation of shorter alanine-rich peptides. As peptide length decreases, residues available for alpha-helix formation also decrease. Short alanine-rich peptides ($n = 1$) in water are not alpha-helical and instead show indications of having a polyproline II-like structure in their far-ultraviolet circular dichroism (CD) spectra [2]. In the presence of solvents such as 2,2,2-trifluoroethanol (TFE) and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), the CD spectra lose the signatures of polyproline II. In HFIP/water mixtures, the spectra suggest that the peptides adopt a partially helical conformation, surprising for such a short sequence. In this work we will discuss the results of CD and Fourier-transform-infrared (FTIR) studies of alanine-rich peptides of varying length. The results suggest that the backbone solvent hydrogen-bonding plays an important role in inducing backbone conformation in small peptides.

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170-Pos Specificity Of The Helical Conformation Induced By 2, 2, 2, Trifluoroethanol

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Board B2

The specificity of helix-induction in the 15-mers of poly-L-glutamic acid and poly-L-lysine by 2,2,2-trifluoroethanol (TFE) was investigated by circular dichroism (CD), NMR, and FTIR at three pH values: 2, 7, and 13. TFE was observed to promote the induction of helical conformation in poly-L-glutamic acid at pH 2.0, and pH 7.0. Similarly, TFE induces helical conformation in poly-L-lysine at pH 7.0 and pH 13. At pH 7.0, the helical conformation was induced in both poly-L-glutamic acid and poly-L-lysine only at higher concentrations of TFE ($>$ 70 % v/v). At lower concentrations of the fluorinated alcohol, very little or no effect was observed in the backbone conformation of the homopolypeptides. ^{1}H-^{15}N HSQC spectra (obtained at ^{15}N natural abundance, at pH 7.0) of the homopolypeptides showed that profound conformational changes occur in the backbone of the polypeptide chains in higher concentrations of TFE. Analysis of the 2D NMR data in conjunction with those obtained using Far UV CD and FTIR revealed that the helix conformation induced at higher concentrations of TFE is non-specific. The results obtained in this study clearly question the validity of structures of short peptides characterized in high concentrations of organic solvents.

171-Pos Effect of Altered Glycosylation on the Structure of the I-like Domain of β1 Integrin: A Molecular Dynamics Study

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Board B3

Glycosylation plays an important role in the regulation of integrin function. Molecular mechanisms underlying the effects of altered glycosylation on β1 integrin structure and function are still largely unknown due to the unavailability of crystal structures of glycosylated integrin. In this study, we used a molecular modeling approach to study the effects of glycosylation on the structure of the I-like domain of the β1 integrin. In particular, we investigated oligosaccharide/peptide interactions using atomically detailed molecular dynamics simulations to observe conformational changes induced by glycosylation. The β1 integrin I-like domain contains three asparagine residues with the appropriate consensus sequence for N-glycosylation. Accordingly, we modeled the I-like domain with all three of these glycans with and without terminal α2–6 sialic acids. Our results demonstrated that the interactions between oligosaccharides and the I-like domain resulted in conformational changes within key functional regions of the I-like domain of β1